ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1977, p. 821-825
Vol. 11, No. 5

Printed in U.S.A.

Renal Pharmacology of Netilmicin

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Received for publication 23 December 1976

Netilmicin (Sch 20569), a new semisynthetic aminoglycoside, was studied for its effects on kidney function and mechanisms by which it is handled by the kidneys. Measurements of glomerular filtration rate (GFR) and urinalysis in chronic rat studies indicated that the nephrotoxicity of netilmicin was remarkably less than that of gentamicin. Gentamicin caused a dose-related reduction in GFR in association with glucosuria and elevated fractional excretion of K^+. By contrast, high doses of netilmicin produced only slight reduction in GFR with increased fractional excretion of K^+ but without glucosuria. In separate experiments, rats were shown to excrete 71 to 90% of netilmicin or gentamicin in 24 h after daily intramuscular administration of doses of 20 or 40 mg/kg for 4 days. In acute experiments on anesthetized dogs, GFR and renal plasma flow were unaffected at serum levels of 11.0 ± 0.6 μg/ml maintained by constant infusion of netilmicin for 5 h. The renal clearance of netilmicin was significantly correlated with GFR. The urinary output of netilmicin was 80.0 ± 4.2% of the infusion rate and was independent of urine flow over the range of 0.04 to 0.33 ml/kg per min. Preferential accumulation of netilmicin occurred in the renal cortex; the cortex-serum and medulla-serum ratios were 9.9 ± 1.2 and 4.2 ± 0.6, respectively. In addition, the extraction ratio of netilmicin, which was lower than that of inulin, suggested that netilmicin reabsorption occurs in the proximal tubule and results in cortical accumulation. It is concluded that netilmicin, like gentamicin, is excreted by the dog kidney by glomerular filtration plus limited reabsorption. However, the new drug is characterized by low intrinsic nephrotoxicity in rats.

Netilmicin (Sch 20569) is a newly described semisynthetic aminoglycoside (18). Its effect on kidney function was evaluated in comparison with gentamicin in rats chronically treated with the drugs. In addition, urinary excretion of the drugs was measured after repeated administration. The renal handling of netilmicin was investigated in acute dog experiments to determine the method of drug excretion. The intrarenal distribution was studied to determine the extent and location of drug accumulation in the dog.

(This work was presented in part at the 16th Interscience Conference of Antimicrobial Agents and Chemotherapy, Chicago, 1976.)

MATERIALS AND METHODS

Renal function study in rats. Seventy-eight male Sprague-Dawley rats (180 to 200 g) were divided into 13 groups of six each. They were injected intramuscularly (i.m.) with saline, gentamicin (batch no. GMC-4M-6231), or netilmicin (batch no. 7733-15) according to the dosing schedule listed in Table 1.

Twenty-four hours after administration of the last dose, the animals were anesthetized by intraperitoneal injection of 100 mg of thiobarbital (Inactin) per kg. Body temperature was maintained at 38°C. After tracheostomy, the left jugular vein was cannulated for blood sampling and the right femoral vein was cannulated for fluid infusion. Carotid arterial blood pressure was measured with a Statham pressure transducer. Urine samples were taken by puncture of the urinary bladder for urinalysis with Labstix (Ames Co., Elkhart, Ind.), and the bladder was then cannulated for urine collection. Priming the animals with 0.4 ml of normal saline containing [^{14}C]inulin (2.4 μCi/ml) was immediately followed by continuous infusion of the same solution at 1.2 ml/h. Collection of blood and urine samples began after allowing 1 h for equilibration. The individual experiments consisted of three consecutive 30-min clearance periods. The [^{14}C]inulin activity in plasma and urine was measured in a liquid scintillation counter with external standardization. The channel ratio method was used to account for quenching.

Urinary excretion study in rats. Twenty-four male Sprague-Dawley rats (180 to 270 g) were kept singly in metabolic cages. The animals were divided into four groups of six each. Netilmicin (batch no. 7733-15) and gentamicin (batch no. GMC-4M-6231) were given by a single daily i.m. injection into the hind limb at a dosage of 20 or 40 mg/kg per day for

TABLE 1. *Incidence of glucosuria and FE_K in rats treated with gentamicin and netilmicin*

Treatment[a]	No. of rats	FE_K[b] (%)	Glucosuria[c]
Saline control			
0.2 ml/rat o.d. × 7 days	6	19.1 ± 2.3	0/6
0.2 ml/rat o.d. × 14 days	6	15.8 ± 3.2	0/6
0.2 ml/rat b.i.d. × 7 days	6	20.3 ± 3.7	1/6[d]
Gentamicin			
80 mg/kg o.d. × 7 days	6	27.1 ± 2.8*	1/6
120 mg/kg o.d. × 7 days	5	68.1 ± 18.5*	4/5
160 mg/kg o.d. × 7 days	3	50.7 ± 14.6	3/5
40 mg/kg b.i.d. × 7 days	5	58.4 ± 26.6	3/6
50 mg/kg b.i.d. × 7 days	6	46.1 ± 10.1*	3/6
60 mg/kg b.i.d. × 7 days	6	45.9 ± 6.2*	2/6[e]
Netilmicin			
160 mg/kg o.d. × 14 days	4	27.0 ± 1.8*	0/4[e]
80 mg/kg b.i.d. × 7 days	5	32.5 ± 1.8*	0/6
125 mg/kg b.i.d. × 7 days	4	24.9 ± 5.3	0/5[d]
170 mg/kg b.i.d. × 7 days	4	45.8 ± 4.8*	0/4[e]

[a] The drugs were given i.m. in saline of a volume similar to that of controls. (o.d.) Once daily; (b.i.d.) twice daily.

[b] All values represent mean ± standard error. (*) Indicates significant difference ($P < 0.05$) from appropriate controls.

[c] Glucosuria determined prior to clearance experiments. Numbers signify number of incidences per number of observations.

[d] One of six animals died from acute toxicity.

[e] Two of six animals died from acute toxicity.

four consecutive days. Urine was collected daily during the first 4 h and from 5 to 24 h after administration of drugs to the rats. To ensure accurate and complete recovery, urinary bladders were emptied by manual pressure on the suprapubic area at the beginning and end of each collection period.

Clearance studies in anesthetized dogs. The experimental procedures were the same as described previously (3). Seventeen mongrel dogs of either sex (mean body weight, 11.3 kg; range, 8.1 to 14.1 kg) were fasted overnight and were anesthetized with 30 mg of nembutal per kg intravenously (i.v.). Clearance rates of *para*-aminohippuric acid (PAH) and inulin were used to measure effective renal plasma flow and glomerular filtration rate, respectively. A 20-mg amount of netilmicin (batch no. 5533-156-I) per kg was given i.v. and followed by sustaining infusion at a rate of 3.0 mg/kg per h, the total dose administered being approximately 200 mg per animal over a period of 5 h. Netilmicin and gentamicin from the preceding studies were assayed by the cylinder-plate method with *Bacillus subtilis* (ATCC 6633) as the test organism (3).

RESULTS

Rats. (i) Renal function study. Frequent occurrences of glucosuria were associated with gentamicin (Table 1). By contrast, glucose was not detected in the urine of netilmicin-treated animals. Glucosuria was noted in one control animal.

The effects of gentamicin and netilmicin on glomerular filtration rate (GFR) are shown in Fig. 1. A dose-related reduction in GFR was observed in gentamicin-treated groups. By contrast, moderate decreases in GFR occurred only when high doses of netilmicin were used. On the basis of dose-response curves, 40% reduction in GFR occurred at a 600-mg/kg total dose of gentamicin compared with a 2,400-mg/kg total dose of netilmicin. Elevation in fractional excretion of K^+ (FE_K) tended to accompany reductions in GFR (Table 1). In general, gentamicin seemed to produce greater rises in FE_K than did netilmicin.

Five of 18 rats receiving the higher doses of netilmicin died from acute toxicity (see Table 1). Hypoactivity, ataxia, and irregular respiration were the principal signs of toxicity. Histopathological work on the kidneys was not performed on any of the animals tested.

(ii) Urinary recovery of netilmicin and gentamicin during repeated administration. On the first day after i.m. administration of 20 or 40 mg of netilmicin per kg, 76.6 ± 4.1 and 69.9 ± 4.9% of the total injected dose, respectively, were excreted in the urine during the first 4 h, followed by 6.5 ± 0.9 and 7.7 ± 0.8%, respectively, during the next 20 h. Similar fractions of the injected drug appeared in the urine after daily treatment through day 4, with a mean of 86% excreted over each of the subsequent 3 days at 20 mg/kg and a mean of 84% at the 40-mg/kg dose. Gentamicin demonstrated a similar ex-

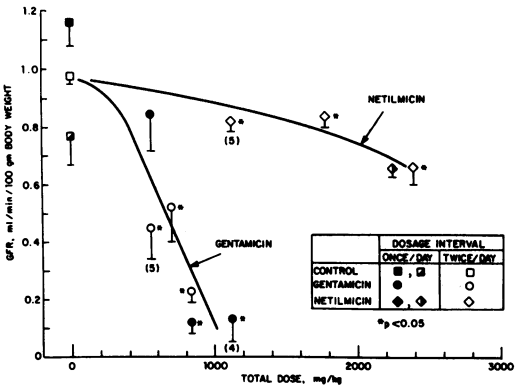

FIG. 1. *Effect of chronic dosing of gentamicin and netilmicin on GFR in rats. Netilmicin and gentamicin are represented by circles and diamonds, respectively. Squares represent appropriate saline controls. Solid symbols denote single daily dose treatment and open symbols represent twice-daily treatment, both for a period of 7 days. The half-open symbols indicate once-daily treatment for 14 days. On the abscissa is the total amount of drug in milligrams per kilogram of body weight given to the animals over the entire treatment period. Each point represents the mean value of individual groups; the vertical bars are standard errors. Control and gentamicin groups consisted of six animals each and netilmicin groups consisted of four animals each, except where indicated by numbers in parentheses.*

cretion pattern, with 24-h excretion of a mean of 79% per day at 20 mg/kg and a mean of 81% at the 40-mg/kg dose.

Dogs. (i) Renal hemodynamics. Fairly steady serum levels of netilmicin (11.0 ± 0.6 μg/ml, $N = 10$) were achieved during the eight consecutive clearance periods (Fig. 2). The inulin and PAH clearances remained stable in the presence of netilmicin. The mean values of the two parameters did not differ from those of controls (Table 2). In addition, the arterial pressure was also found to be stable throughout these acute experiments.

(ii) Renal clearance of netilmicin. The mean clearance value of netilmicin was slightly lower than the mean inulin clearance in the 10 dogs studied ($P < 0.05$, paired comparison) (Table 2). Based on the finding that the ratio of netilmicin clearance to inulin clearance (0.92 ± 0.03) was significantly less than unity ($P < 0.05$), it appeared that a small fraction of netilmicin was reabsorbed by the renal tubule after being filtered. With serum levels maintained at a steady state, the netilmicin appeared in the urine at 80.0 ± 4.2% of the constant infusion rate. Both the fractional excretion and absolute excretion rates were independent of variations in urine flow rate (Fig. 3).

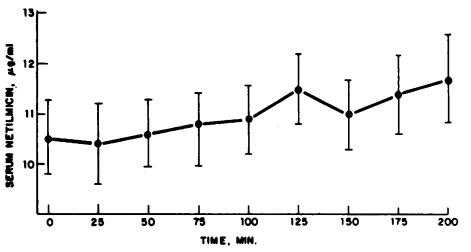

FIG. 2. *Serum levels of netilmicin in anesthetized dogs. Netilmicin was given at 2 mg/kg i.v. and followed by a sustaining infusion at a rate of 3.0 mg/kg per h. Collection of blood and urine samples began 90 min after start of infusion. Each point represents the mean of 10 animals; the vertical bars are standard errors.*

TABLE 2. *Renal handling of netilmicin in anesthetized dogs*[a]

Determination	Saline group ($N=7$)	Netilmicin group ($N=10$)
C_N (ml/min)[b]		43.3 ± 2.9
C_{In} (ml/min)[b]	46.7 ± 4.4	47.5 ± 3.8
C_{PAH} (ml/min)[b]	108.0 ± 6.0	121.0 ± 13.0
FE_N (%)[c]		92.4 ± 3.4[d]
U_NV (μg/kg per min)[e]		40.0 ± 2.1
V (ml/kg per min)[e]	0.14 ± 0.03	0.18 ± 0.03

[a] All values represent mean ± standard error.

[b] C_N, C_{In}, and C_{PAH} represent clearance values for netilmicin, inulin, and PAH, respectively.

[c] FE_N represents the percentage of filtered netilmicin being excreted in the urine.

[d] The mean value is significantly ($P < 0.05$) less than 100.

[e] U_NV represents the absolute rate of urinary excretion of netilmicin. V represents the urine flow rate.

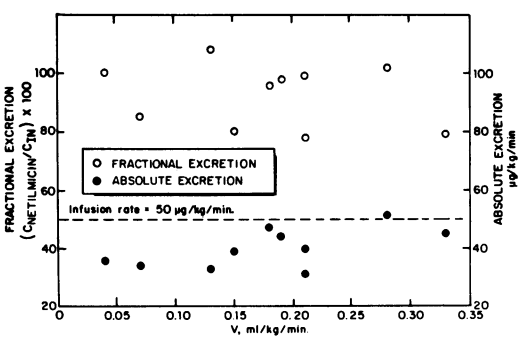

FIG. 3. *Urine flow rate versus urinary excretion of netilmicin in anesthetized dogs. Symbols: (○) Fractional excretion of netilmicin, 8 out of 10 are below 100%. (●) Absolute rate of excretion, of which all except one were less than the sustaining infusion rate (- - -). Each circle represents the mean of eight consecutive clearance values per animal.*

(iii) Renal extraction ratios. The extraction ratio (Table 3) and clearance value of PAH (Table 2) in netilmicin-treated dogs did not differ from controls, indicating that the drug did not interfere with the tubular secretion of PAH. On the other hand, the extraction ratio of netilmicin was significantly lower than that of inulin. This finding provided additional evidence that the drug undergoes tubular reabsorption.

(iv) Renal tissue and urine antibiotic levels. Accumulation of netilmicin occurred in the renal cortex; the mean cortical concentration was 10 times that of the steady-state serum level (Table 4). On the other hand, the renal medulla had a much lower level despite the high values of netilmicin present in the urine.

DISCUSSION

Aminoglycoside antibiotics are promptly excreted in the urine mainly by glomerular filtration in man (5) and experimental animals (2, 16). Close similarity in the excretion patterns of netilmicin and gentamicin was demonstrated in this study. Rapid elimination of the two drugs was consistent with their short serum half-life in rats, i.e., 0.5 h (11; G. Miller, unpublished data).

The urinary excretion of netilmicin is also rapid in dogs. With the serum levels maintained in a steady state, the output of the drug in an active form was 80% of the infused dose. Apparently, disposition of the drug is associated with little metabolism; no metabolites of the drug have yet been identified. Furthermore, the renal clearance of the drug was found to be directly related to glomerular filtration (Fig. 4). The calculations of netilmicin clearance were based on the assumption that the new drug, like gentamicin (6, 13, 14), has negligible plasma binding. Regardless, the significant relationship between netilmicin and inulin clearances would remain unaffected even if protein binding did occur. Variations in urine flow within the ranges found in the present study did not appear to influence the excretion of netilmicin. Gentamicin had previously been shown to exhibit the same behavior in dogs (3) and in man (9).

TABLE 3. *Extraction ratios of netilmicin, inulin, and PAH in anesthetized dogs*[a]

Drug	Saline group (N=7)	Netilmicin group[b] (N=9)
Inulin	0.29 ± 0.05[c]	0.31 ± 0.02
PAH	0.60 ± 0.03	0.76 ± 0.02
Netilmicin		0.16 ± 0.03[d]

[a] Extraction ratio = [Pa − Pv]/Pa, where Pa and Pv are the femoral arterial and renal venous concentrations of the individual compounds, respectively. All values represent the mean ± the standard error.

[b] Due to proximity of the confluence of the renal vein and spermatic or ovarian vein to the inferior vena cava, determinations of extraction ratio were not performed in one of the 10 netilmicin dogs.

[c] Mean value of four animals. Measurements of venous inulin in the other three animals were not done.

[d] Significantly different from the mean extraction ratio for inulin ($P < 0.05$).

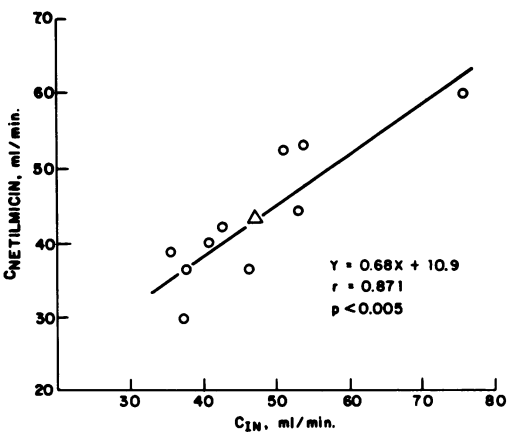

FIG. 4. *Relationship of netilmicin clearance (C_N) to inulin clearance (C_{In}). The linear regression line relates C_N to C_{In}. Each point represents a mean value of eight consecutive clearance values per animal, and the triangle depicts the mean of C_N average in relation to the C_{In} average.*

TABLE 4. *Netilmicin in serum, kidney, and urine of anesthetized dogs*[a]

Concentration				Ratio[b]		
Tissue[c] (μg/g)		Liquid (μg/ml)		Cortex/serum	Medulla/serum	Cortex/medulla
Cortex	Medulla	Serum	Urine			
102.4 ± 7.2	45.9 ± 6.6	11.0 ± 0.6	338.0 ± 87.0	9.9 ± 1.2	4.2 ± 0.6	2.6 ± 0.4

[a] All values represent mean ± standard error of 10 dogs.

[b] Serum concentrations of netilmicin are shown in Fig. 2.

[c] Tissue concentration is based on the wet weight of tissues.

The rat has served as a useful species in studying the potential nephrotoxicity of many compounds including aminoglycosides (10, 12). A. Sugarman et al. (Clin. Res. 24:413A, 1976) demonstrated that gentamicin caused a linear, dose-related decrease in rat GFR. In our study, the renal impairment caused by gentamicin was characterized by a reduction in GFR in association with glucosuria and potassium wasting as reflected by elevation in the fractional excretion of potassium. By contrast, the rat was quite insensitive to netilmicin with reference to kidney function. The daily doses of netilmicin utilized in evaluation of nephrotoxicity were only limited by its relatively high acute toxicity (mean lethal dose, ca. 200 mg/kg, i.m.) in comparison with gentamicin. Nevertheless, our data indicate that the nephrotoxicity of netilmicin was clearly less than that of gentamicin.

High concentrations of netilmicin similar to that described for gentamicin (3) occurred in the renal cortex. Prolonged accumulation in the cortex (11) may be related in some way to the nephrotoxicity typically associated with aminoglycoside nephrotoxicity in experimental animals (8). However, conclusive evidence in support of this view is not yet available. In this regard, cephaloridine also caused similar lesions in the kidney, which suggests that its nephrotoxicity was related to high intracellular concentrations of the drug (17). Despite the similarity of cortical uptake of netilmicin and gentamicin, the former drug has remarkably less effect on kidney function, probably due to its lower intrinsic toxicity or different kinetics in binding to renal tissues.

The renal extraction ratios of netilmicin and gentamicin (3) are similar, and both are lower than those of inulin and PAH. The results indicate that a small fraction of filtered drug is reabsorbed by the proximal tubule since the bulk of drug is recoverable in the urine. Previous clearance studies in man and dogs have indeed shown that limited tubular reabsorption is associated with gentamicin (1, 3, 7), tobramycin (15), and amikacin and kanamycin (4). It appears that the reabsorptive mechanism present in the epithelium of proximal tubules is responsible for the cortical buildup of netilmicin and the other aminoglycosides.

In conclusion, whereas netilmicin demonstrated lower nephrotoxicity than gentamicin, the excretory mechanisms that mediate the renal handling of the two drugs are essentially the same. Rapid excretion can be ascribed to their primary dependence on glomerular filtration with limited tubular reabsorption.

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